Cellaxess® ACE

Neuronal and differentiated cell lines

Cell types:
SH-SY5Y,
N2a and
PC12

System:
Cellaxess ACE

Efficient transfection of neuronal cell lines in 96 well plate

Transfection of differentiated cells with preserved transfection efficiency and viability

Transfection of differentiated neuronal cell lines with fully developed neuronal processes

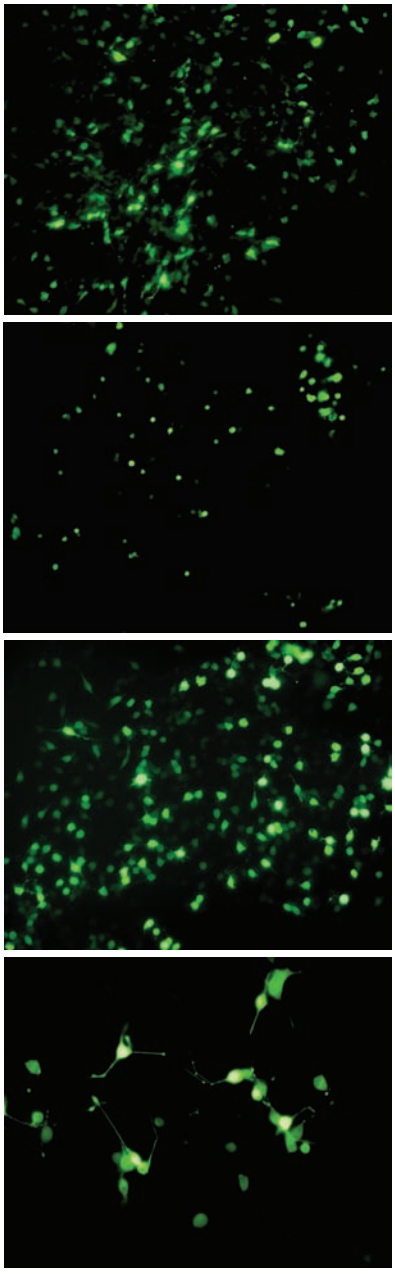
Introduction

Neuronal cell lines are often used in neuroresearch as complementary model systems to study neuronal differentiation, neurochemical metabolism, neuronal protein expression and cancer. One important quality of several neuronal cell lines is the possibility to initiate differentiation to increase the physiological relevance of the cells.

Transfection of differentiated neuronal cell lines is known to be difficult because of the increased sensitivity of differentiated cell. Additionally the transfection needs to be performed on adherent cells which is not possible with other electroporation techniques.

In the results we show transfection of several neuronal cell lines and differentiated PC12 cells. The transfection efficiency for differentiated PC12 cells was comparable to non differentiated cells.

With these results we prove that the Cellaxess ACE is an excellent tool in neuronal research based on neuronal cell lines. It can be used for a variety of cell lines with comparable results and it gives access to transfection of differentiated cells with fully developed neurite extensions.



Results

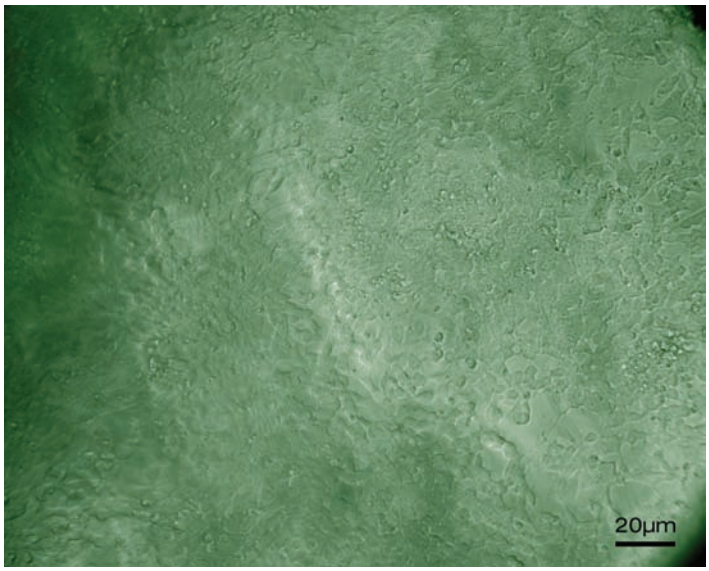
The cells were evaluated for GFP expression with fluorescence microscopy 24 h after transfection. Cell viability was evaluated with fluorescence microscopy 24 h after transfection by propidium iodide staining compared to non treated controls. The differentiated PC12 cells clearly expressed GFP after 24 h within the cell body and all along the cell processes. The expression increased during the first 48 h and was stable up to 7 days after transfection.

Figure 1

SH-SY5Y 24h after transfection with pCopGFP-C vector

Transfection efficiency: 25%

Viability: 70%



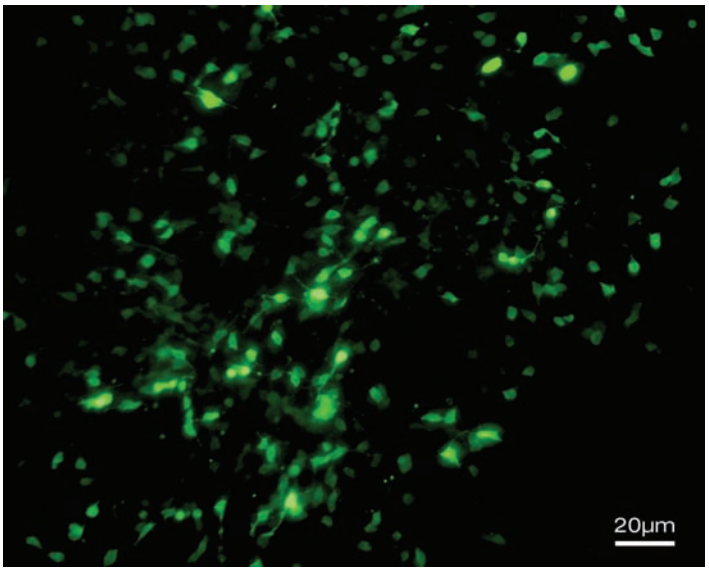
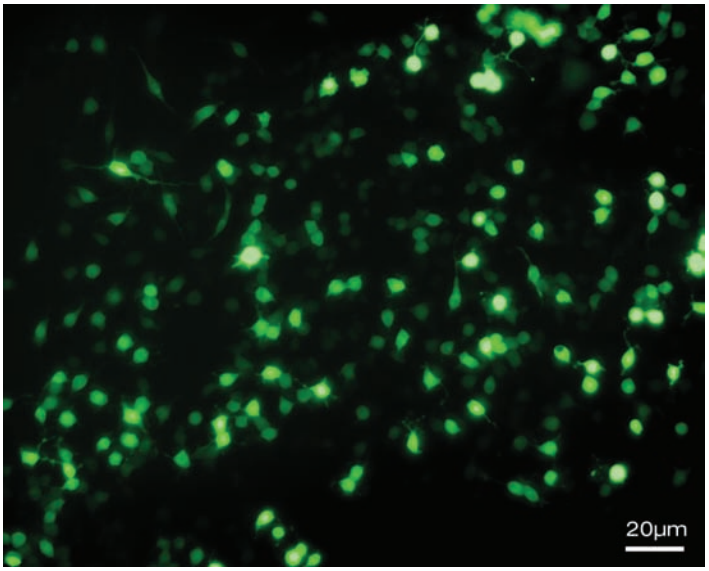


Figure 2

N2a 24h after transfection with pCopGFP-C vector

Transfection efficiency: 23%

Viability: 95%



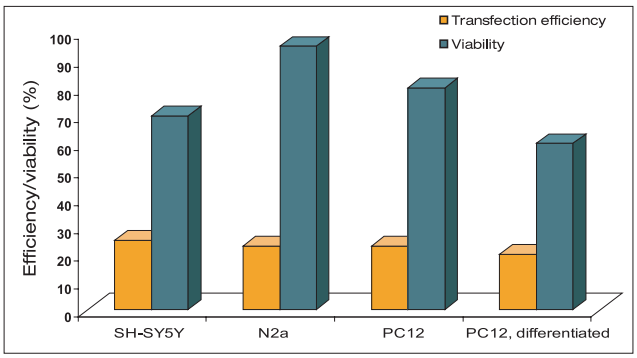
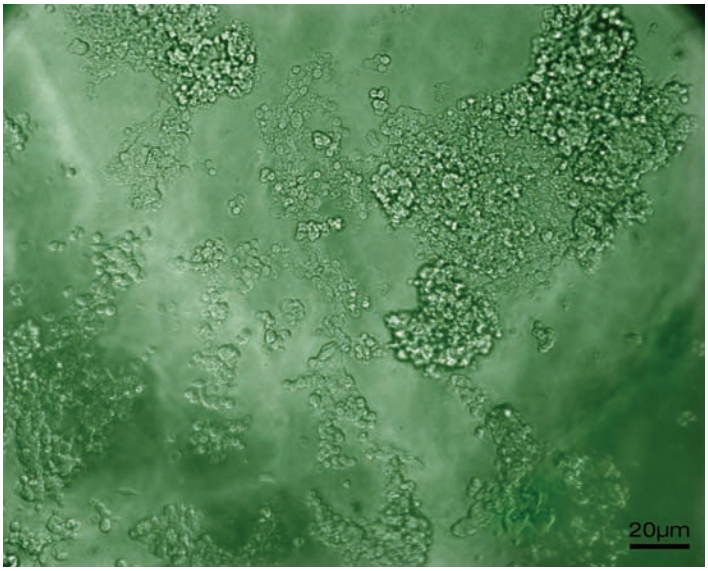


Figure 3

PC12 24h after transfection with pCopGFP-C vector

Transfection efficiency: 23%

Viability: 80%



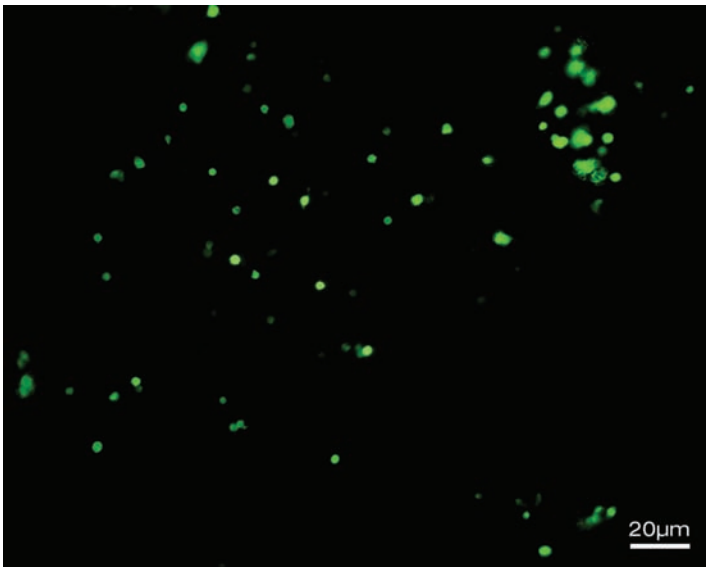
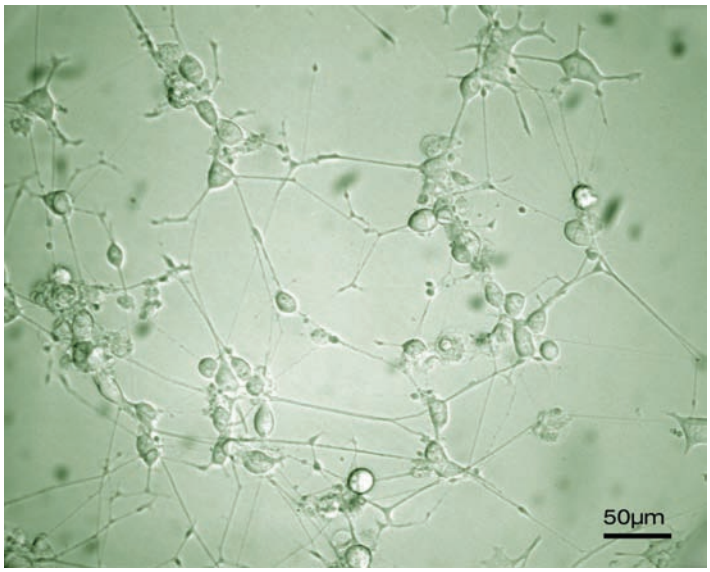


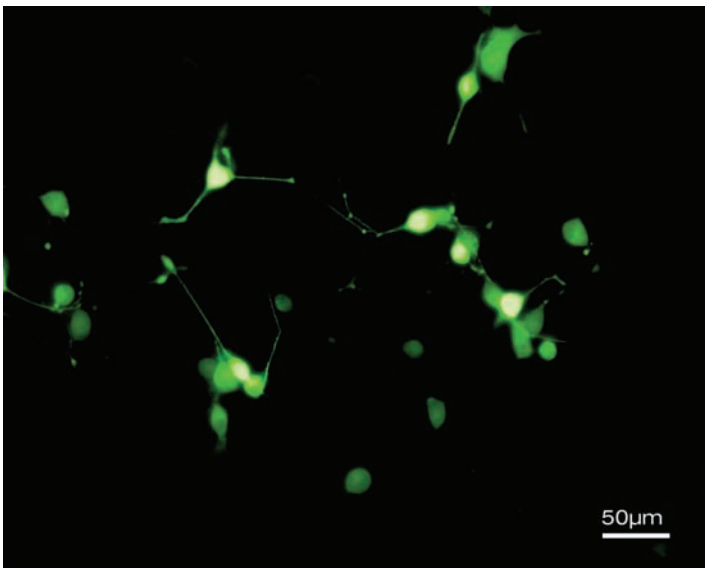
Figure 4

Differentiated PC12 24h after transfection with pCopGFP-C vector

Transfection efficiency: 20%

Viability: 60%





Transfection with Cellaxess ACE system

The culture dish or plate containing the cells to be transfected was removed from the incubator. The transfection solution containing the pCopGFP vector (Eurogen) at 20-50 ng/µl final concentration was pipetted into the ACE capillary by means of a standard p100 micropipette. The dish was positioned under the ACE module, and the capillary was lowered into the dish. After an approximated 15 second wait time, the pre-programmed transfection protocol was executed. The capillary was raised, the plate or dish was temporarily removed, and remaining solution was drained by blowing through the capillary using a p1000 micropipette. After this, the capillary was once again filled with plasmid solution, and the next transfection was carried out. Once the desired number of transfections had been carried out, the dish or plate was returned to the incubator.

Cell culture

Cells lines were acquired from ATCC. Cells were cultured in 96 well plate in the medium recommended by the supplier. The cells were from passage number 5-15. SHSY-5Y and N2a cells were cultured for 2-4 days to a final confluence of 60-80% and PC12 cells were cultured on collagen coated wells for 3-5 days to a confluence of 60-70%. Differentiated PC12 cells were cultured in 35 mm dishes coated with collagen and maintained in medium with NGF (50 ng/ml) and low serum content for 7 days prior to transfection.

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